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Amino Acid Analysis of the Isolated and Purified Components from Crystalline Toxin of *Clostridium botulinum* Type A

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The toxic and hemagglutinating components of the crystalline toxin of *Clostridium botulinum* were analyzed for their amino acid content and were proven to be completely different proteins.

That the crystalline preparations of the toxin of *Clostridium botulinum* may contain material not related to toxicity was recognized by a number of investigators: Lamanna (6), Lamanna and Lowenthal (7), Stefanye et al. (11), and Wagman (13). Recent work has shown that crystalline type A toxin is indeed a mixture of proteins. DasGupta et al. (5) found that the toxin contained two proteins referred to as the α and β components. The α component had a molecular weight of 150,000, comprised only 20% of the protein based on ultracentrifuge data [Boroff et al. (1)], and had virtually all of the toxicity of crystalline toxin. The bulk of the remaining protein was the β component which had hemagglutinating properties and was found to be an aggregate of 250,000-molecular-weight subunits (5). The α component has been found to be homogeneous by anion (3-5) and cation exchange chromatography (4), on gel filtration column (3), by immunoelectrophoresis (5), Ouchterlony gel double-diffusion technique (5), and by sedimentation equilibrium technique (1).

The separation of crystalline botulinum toxin into two major components indicates that existing data on amino acid composition of toxin may be irrelevant to the amino acid composition of the α or β components. Significant differences in the chemical composition of α and β would confirm that they are completely different proteins. Consequently, this work was undertaken to investigate this point. In addition, the data obtained may be of value for future comparison with similar components isolated from other serological types of toxin.

The amino acid analyses were carried out on α and β components isolated by diethylaminoethyl (DEAE)-cellulose chromatography as previously described (5). Crystalline toxin, kindly supplied by E. J. Schantz, Fort Detrick, Frederick, Md., had a specific toxicity of 2.4×10^8 LD_{50}/mg of N for 20-g white mice by intraperitoneal injection.

Protein was determined by two methods: (i) that of Lowry et al. (8) by using crystalline serum albumin as a standard, and (ii) by summation of the calculated partial weights of each amino acid in the hydrolysate by using the data obtained from the amino acid analysis. Protein was hydrolyzed in 6 N HCl at 105 C. The general method described by Moore and Stein (10) was used in preparing the samples for hydrolysis which, in all cases, was carried out for 36, 60, and 84 hr. Amino acid values shown are the mean of three determinations, excepting threonine and serine whose concentrations were obtained by extrapolation to zero hydrolysis time assuming first order kinetics (10). Tyrosine values were constant throughout the hydrolysis time period and were not corrected to zero time. Cysteine and cystine data as cysteic acid were obtained in parallel experiments by using performic acid-oxidized protein samples (9) which were hydrolyzed for 75 to 80 hr.

Analyses were carried out on a Spinco (model 120 C) amino acid analyzer. Long light-path cuvettes and a 4- to 5-mv recorder measuring circuit were used to increase sensitivity. Peak heights were measured by using an overlay supplied by Beckman Instruments, Inc. Tryptophan determinations were made on intact protein by the method of Spies and Chambers (12).

The amino acid composition of the α and β components is shown in Table 1. The data are in terms of micromoles of amino acid per 100,000 μg of protein. The value for protein concentration

TABLE 1. *Amino acid composition of α and β components isolated from crystalline botulinum type A toxin*

Amino acid	Amt of amino acid (μmole/10^3 μg of protein)	
	α	β
Aspartic acid	126.8	163.0
Threonine	61.2	50.5
Serine	98.0	79.1
Glutamic acid	114.0	92.4
Proline	20.3	28.5
Glycine	65.8	85.1
Alanine	49.0	53.7
Valine	53.8	41.6
Methionine	9.9	57.7
Leucine	64.2	77.4
Isoleucine	73.2	71.6
Tryosine	31.0	27.2
Phenylalanine	42.0	33.5
Cysteine + cystine as cysteic acid	9.2	14.7
Lysine	67.5	59.8
Histidine	10.3	11.5
Arginine	27.5	24.4
Tryptophan[a]	4.0	8.5

[a] Tryptophan content was determined on the unhydrolized toxin by the Spies and Chambers method (12).

shown in the table was determined by the second method described above, assuming quantitative recovery of the amino acids. It was found that 1.00 mg of protein, as determined by method (i) calculates to 0.450 and 0.865 mg of protein by using method (ii), for the α and β components, respectively. The agreement of the two methods for the β component is reasonable. However, the two methods show a large discrepancy for the α component. Since the α component as isolated from DEAE-cellulose is very dilute, the protein value obtained with the Lowry et al. (8) method could be subject to a large error. It is of interest to note that the protein value for the α component calculated from Table 1 suggests that α may comprise only 10% of the crystalline toxin protein instead of the 20% previously reported (1).

The data in Table 1 also show that the α and β components exhibit differences in amino acid composition. The molecular weight of α would be a multiple of 25,000 and that of β a multiple of 11,800. Referring to previous molecular weight values (1, 3), the data of Table 1 would yield a calculated molecular weight of 150,000 for α and 248,000 to 259,000 for β, based on the tryptophan content of each component. No attempt was made to determine amide content in the experiments. However, if all of the aspartic and glutamic acid occurred as the amide, the molecular weight of α would be only slightly decreased.

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